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**TASK ORDER FINAL REPORT FOR THE PERIOD
20 JANUARY 2003 TO 19 JANUARY 2005**

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14. ABSTRACT The goal of the reviewed research is to determine the effect of laser radiation on human tissues. The analysis includes quantification of cellular and tissue parameters, response to laser irradiation and modeling of the laser-tissue interaction. The research encompassed photoacoustic, photothermal, cellular insult, photochemical and photomechanical processes and their effect on in vitro and in vivo models. Understanding laser tissue interaction is the first step toward optimizing military application of laser radiation. The research review emphasizes the Occupational and Environmental Health of laser tissue interaction, with experiments and modeling efforts which result in suggestions to the laser safety community where safety standards either do not exist, or where deficiencies in biological data has made the criteria for setting standards ambiguous. The laboratory offers extensive laser facilities and support equipment for in depth analysis of laser tissue interaction.					
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1. Summary of accomplishments

Contract: F41624-02-D-7003, Order 001 - Optical Radiation Bioeffects
TO1 Final Report, Data Item A014

Northrop Grumman, as part of the Laser Bioeffects Integrated Product Team, completed work in its second two-year term as an AFOSR "STAR Team." The Team's interaction has continued with researchers from Wellman Laboratory of Harvard Medical School, The Uniformed Services University of the Health Sciences, the University of Texas Health Sciences Center in San Antonio, The US Air Force Academy, Florida International University, Ft. Hays State University, The University of Illinois-Champaign/Urbana and the Lübeck Medical Laser Center in Lübeck, Germany.

Cognizant of future AF scientific needs, the Laser Bioeffects Team has turned their attention toward the biomolecular effects of lasers on the eye and skin. The impact of lasers will be of greatest concern to commanders who will see applications from advanced visual displays (e.g. retinal displays) to high-energy laser weaponization. This has been emphasized at numerous conferences and taskings (including SECDEF briefing on laser safety). By directing our attention toward the science behind the problems that will be faced in the modern battlefield, we will protect our personnel and allow the effective use of advanced DoD laser systems.

The Northrop Grumman Laser Bioeffects Team successfully validated an "*in vitro*" model of pigmented RPE cell culture that allows for reproducible variation of pigmentation. Pigmentation of cultured hTERT-RPE1 cells is achieved by application of aliquots of previously isolated bovine melanosomes. The modification depends on the high efficiency of the RPE cells for internalizing the pigment by a phagocytic mechanism.

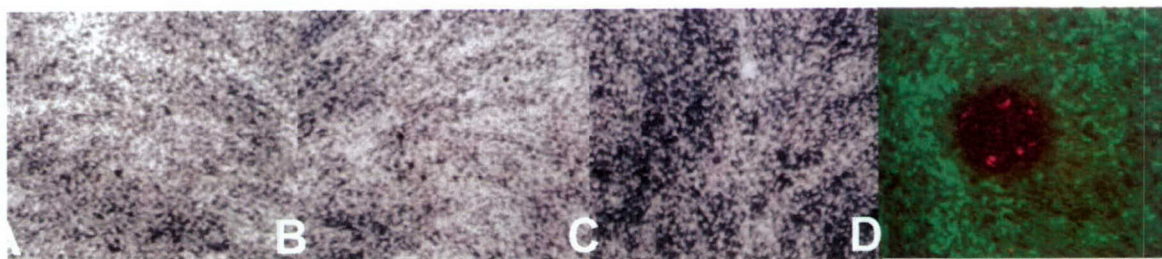


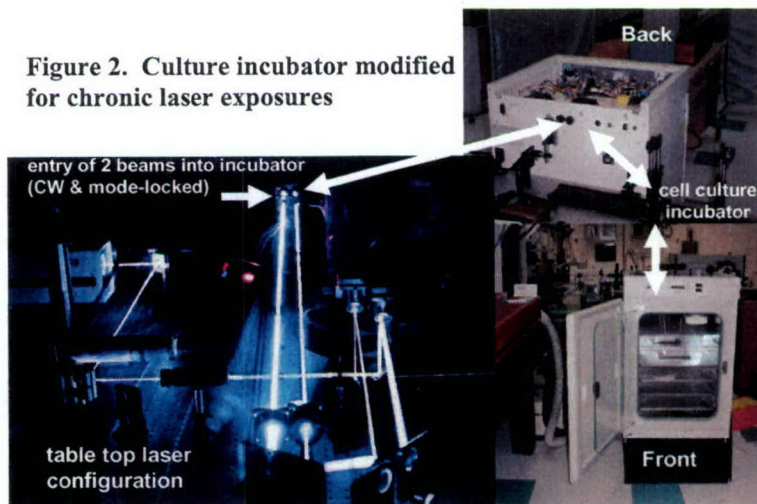
Figure 1. Artificially pigmented RPE cells as laser bioeffects model. Human hTERT-RPE1 cells have increasing degrees of pigmentation (A-C) by simply adding increasing volumes of bovine melanosome stock solutions. In combination with fluorescence "viability" indicator dyes the artificially pigmented cultures are useful in assessing laser damage, such as the acute (0.25-s) 810-nm mode-locked laser damage region (red color) shown in panel D.

Damage thresholds for acute (0.25 sec) mode-locked and CW laser exposures at 810 nm in our artificially pigmented cell system approximated trends seen in previous *in vivo* measurements, thus validating the new model system. At $3,000 \text{ W cm}^{-2}$ (750 J cm^{-2}) and above, pigmented cells underwent a cavitation-mediated overt cell disruption. We were unable to produce identifiable fluorescent targets of cell death (4 hr or 24 hr post-exposures of 0.25 sec) at lower fluences, regardless of the degree of RPE pigmentation. In the absence of pigmentation, hTERT-RPE cell

death was not detected (fluorescence) in cultures exposed (0.25 sec) to fluences as high as $30,000 \text{ W cm}^{-2}$ ($7,500 \text{ J cm}^{-2}$) at time points of 1 hr, 4 hr, 24 hr, or 48 hr post-exposure. These measurements allow us to validate the levels required for thermal damage, thereby validating the use of this human cell line for laser-tissue interaction studies without the use of nonhuman primates.

Photochemical oxidation as the result of NIR laser exposure in the hTERT-RPE cell line (no pigmentation) was detected and characterized. Using the oxidation-indicating dye CM-H₂DCF-DA at 100 mM, we detected targets of photo-oxidation using 810 nm mode-locked (but not CW) laser exposures. At 810 nm we found a defined transition from no fluorescent target to positive fluorescent target when total radiant exposure (J cm^{-2}) delivered to the cells reached 194,000, regardless of mode-locked exposure duration, as long as the average irradiance (W cm^{-2}) was greater than 10,000. We were unable to generate a fluorescent target using 810 nm CW exposures with radiant exposures as high as $3,300,000 \text{ J cm}^{-2}$ and average irradiances of $33,000 \text{ W cm}^{-2}$ using conditions otherwise identical to those used for mode-locked exposures. We extended our analysis of laser facilitated cellular oxidation to two additional NIR wavelengths. When combined, the photo-oxidation data at 750, 810, and 900 nm show an important action spectrum. The efficiency of the photochemical reaction, as measured by the lowest average irradiance (mode-locked beams) leading to an oxidation target, is wavelength dependent. The 750 nm laser was most efficient requiring approximately $2,000 \text{ W cm}^{-2}$ to generate oxidation zones, followed by 900 nm at about $4,400 \text{ W cm}^{-2}$, with 810 nm at the high end of the range at $9,900 \text{ W cm}^{-2}$.

The Laser Bioeffects Team made use of a novel cell culture incubator modified for laser exposures. The modification has allowed for entry and delivery of two separate laser beams to two separate wells of a microtiter plate while providing an environment conducive for cell survival during extended times of exposure. Exposures at 810 nm and 457 nm have been delivered, with direct comparisons of mode-locked and CW beams because of concurrent delivery.



The bioeffects team acquired a 1315 nm, nanosecond laser to provide the underpinnings for laser safety for the Airborne Laser Program. The bioeffects project was funded by our in-house 6.3 high-energy laser program, but the modeling for this effort was being worked by the basic research component. Minimum visible lesions have been created in both retinal and corneal tissues at this wavelength. These were created at energies much higher levels than the maximum permissible exposure levels, lending support to relaxing safety standards in this regime, giving relief to the ABL program. Dr. Joe Zuclich of Northrop Grumman is proposing new maximum

permissible exposure (MPE) levels for the entire near-infrared wavelength regime based on the work done in our group. These new MPE's would dramatically affect the hazard evaluations being done for novel DoD laser applications.

Table 1. Current and proposed MPEs. For exposure duration $10^{-9} - 50 \times 10^{-6}$ s.

Wavelength (μm)	Exposure Duration (s)	Current MPE ($\text{J}\cdot\text{cm}^{-2}$)	Proposed MPE ($\text{J}\cdot\text{cm}^{-2}$)
1.05 - 1.15	$10^{-9} - 50 \times 10^{-6}$	5×10^{-6}	5×10^{-6}
1.15 - 1.20	$10^{-9} - 50 \times 10^{-6}$	$5 \times 10^{18(\lambda - 1.15)} \times 10^{-6}$	$5 \times 10^{18(\lambda - 1.15)} \times 10^{-6}$ (0.158 at $\lambda = 1.4 \mu\text{m}$)
1.20 - 1.40	$10^{-9} - 50 \times 10^{-6}$		
1.15 - 1.40	$10^{-9} - 50 \times 10^{-6}$		
1.40 - 1.50	$10^{-9} - 50 \times 10^{-6}$	0.1	
1.50 - 1.80	$10^{-9} - 50 \times 10^{-6}$	1.0	
1.80 - 2.60	$10^{-9} - 50 \times 10^{-6}$	0.1	
2.60 - 10^3	$10^{-9} - 10^{-7}$	0.01	
2.60 - 10^3	$10^{-7} - 50 \times 10^{-6}$	$0.56 t^{0.25}$	
1.40 - 1.50	$10^{-9} - 50 \times 10^{-6}$		$0.158 + \frac{1 - 0.158}{0.1}(\lambda - 1.40)$
1.50 - 2.60	$10^{-9} - 10^{-7}$		$1.0 - \frac{1 - 0.01}{1.1}(\lambda - 1.50)$
1.50 - 2.60	$10^{-7} - 50 \times 10^{-6}$		$1.0 - \frac{1 - 0.56 t^{0.25}}{1.1}(\lambda - 1.50)$

The Laser Bioeffects Team completed a series of measurements of the damage threshold to skin for 1540 nm and 1315 nm laser pulses. The spot size has been varied for the 1540 nm, microsecond laser exposures up to 0.7 mm. This was increased to several millimeters with the acquisition of a multi-Joule laser. The new 1315 nm laser was used to complete the damage mechanism work necessary for skin safety hazard determination. Several computer models were exercised to predict temperature rises and damage thresholds for the measured thresholds, and a Northrop Grumman subcontract collaborating with the University of Texas and HEDO has measured the time-dependent temperature distribution for 1540 nm laser exposure to help validate our modeling efforts.

The first-ever exposures of skin simulants to terawatt laser pulses were completed. The laser system was a joint-funded 6.1/6.2 laser system which will allow for the evaluation of advanced laser applications using high peak power laser pulses.

Dr. Clarence Cain of Northrop Grumman began writing a manuscript "Sub-50-fs Laser Retinal Damage Thresholds in Primate Eyes with Group Velocity Dispersion, Self-Focusing and Low-Density Plasmas." This paper described the determination of the effects of group velocity dispersion on the retinal damage threshold for pulse durations below 100 fs. In addition, Dr.

Clarence Cain presented an invited tutorial on laser safety with ultrashort lasers at the 2003 BiOS Conference. This was recognition of the pioneering ultrashort pulse, laser-tissue interaction work done within the AFOSR-supported effort.

The Advanced Ultrashort Laser Bioeffects Team furthered our collaboration with the modeling group from the physics department at Ft. Hays State University (Kansas). Dr. Gavin Buffington is the director of the group and has created the FHSU High Performance Computing Facility, which is a parallel-computing system, developed with NASA funding. The work focused on theoretical calculations of chirped-pulse LIB thresholds which were incorporated into a peer-reviewed publication (submitted) on retinal damage for 40 fs laser exposures. The models will be exercised to allow assessment of retinal damage to allow extension of retinal MPEs to the shortest possible value (5 fs).

2. Publications

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